WEST Search History

DATE: Wednesday, February 26, 2003

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,JPAB,DWPI; PLUR=YES; OP=ADJ		
L16	l14 and L15	4	L16
L15	terminator near3 (nos or 35S)	1084	L15
L14	L13 and binary and l3	25	L14
L13	l11 or L12	547	L13
L12	l1 and LB and RB	230	L12
L11	l1 and left border and right border	437	L11
L10	L9 and l7	1	L10
L9	(35S CaMV or ep35S CaMV or pea plastocyanin or high molecular weight glutenin or HMWG or CsVMV or cassava mosaic virus or CoYMV or Commelina yellow mosaic virus) near3 promoter	414	L9
L8	L7 and 35S CaMV promoter	1	L8
L7	L6 and l3	30	L7
L6	l1 and (left border and right border)	437	L6
L5	l1 same l3	73	L5
L4	l1 and L3	84	L4
L3	trfA	124	L3
L2	trfA locus	1	L2
L1	T-DNA	2593	L1

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NEWS 6 Apr 22  Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
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NEWS 11 Jun 10  PCTFULL has been reloaded
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NEWS 13 Jul 22  USAN to be reloaded July 28, 2002;
          saved answer sets no longer valid
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NEWS 19 Aug 19  Aquatic Toxicity Information Retrieval (AQUIRE)
          now available on STN
NEWS 20 Aug 19  IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21 Aug 19  The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22 Aug 26  Sequence searching in REGISTRY enhanced
NEWS 23 Sep 03  JAPIO has been reloaded and enhanced
NEWS 24 Sep 16  Experimental properties added to the REGISTRY file
NEWS 25 Sep 16  CA Section Thesaurus available in CAPLUS and CA
NEWS 26 Oct 01  CASREACT Enriched with Reactions from 1907 to 1985
NEWS 27 Oct 21  EVENTLINE has been reloaded
NEWS 28 Oct 24  BEILSTEIN adds new search fields
NEWS 29 Oct 24  Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 30 Oct 25  MEDLINE SDI run of October 8, 2002
NEWS 31 Nov 18  DKILIT has been renamed APOLLIT
NEWS 32 Nov 25  More calculated properties added to REGISTRY
NEWS 33 Dec 02  TIBKAT will be removed from STN
NEWS 34 Dec 04  CSA files on STN
NEWS 35 Dec 17  PCTFULL now covers WP/PCT Applications from 1978 to date
NEWS 36 Dec 17  TOXCENTER enhanced with additional content
NEWS 37 Dec 17  Adis Clinical Trials Insight now available on STN
NEWS 38 Dec 30  ISMEC no longer available
NEWS 39 Jan 13  Indexing added to some pre-1967 records in CA/CAPLUS
NEWS 40 Jan 21  NUTRACEUT offering one free connect hour in February 2003
NEWS 41 Jan 21  PHARMAML offering one free connect hour in February 2003
NEWS 42 Jan 29  Simultaneous left and right truncation added to COMPENDEX,
          ENERGY, INSPEC
NEWS 43 Feb 13  CANCERLIT is no longer being updated
NEWS 44 Feb 24  METADEX enhancements
NEWS 45 Feb 24  PCTGEN now available on STN
NEWS 46 Feb 24  TEMA now available on STN
NEWS 47 Feb 26  NTIS now allows simultaneous left and right truncation
NEWS 48 Feb 26  PCTFULL now contains images

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=> s trfA
L1        351 TRFA

=> s T-DNA
L2       5371 T-DNA

=> s l1 and l2
L3          1 L1 AND L2

=> d bib abs

L3  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2003 ACS
AN  2001:185905  CAPLUS
DN  134:232870
TI  Minimal vectors derived from pBin19 for use in the transformation of
    plants and their construction and use
IN  Gruber, Veronique; Comeau, David
PA  Meristem Therapeutics, Fr.
SO  PCT Int. Appl., 191 pp.
    CODEN: PIXXD2
DT  Patent
LA  English
FAN.CNT 1
    PATENT NO.      KIND  DATE         APPLICATION NO.  DATE
    --------------  ----  -----------  ---------------  --------
PI  WO 2001018192   A2    20010315     WO 2000-IB1243   20000904
    WO 2001018192   A3    20010920
        W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
           CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
           HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
           LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
           SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
           YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
        RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
           DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
           CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
    FR 2798139      A1    20010309     FR 1999-11112    19990903
    EP 1144608      A2    20011017     EP 2000-954825   20000904
    EP 1144608      A3    20011219
        R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
           IE, SI, LT, LV, FI, RO
PRAI FR 1999-11112    A   19990903
    WO 2000-IB1243    W   20000904
AB  Plant transformation vectors derived from pBin19 that have the
    nonessential sequences removed and that are therefore smaller and more
    useful for plant transformation (clean vectors) are described. The
    invention also relates to a procedure for obtaining these vectors as well
    as transgenic plants contg. them. The vectors contain a plasmid origin of
    replication from pRK2 or a ColE1 plasmid, ***T*** - ***DNA*** ,
    plasmid replication functions, selectable markers for use in bacterial and
    plant hosts and a multicloning site. Construction of a no. of vectors is
    described.


=> d his

    (FILE 'HOME' ENTERED AT 15:51:26 ON 26 FEB 2003)

    FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 15:53:45 ON 26 FEB 2003
L1        351 S TRFA
L2       5371 S T-DNA
L3          1 S L1 AND L2

=> s l2 and ((left border and right border) or (LB and RB))
L4         73 L2 AND ((LEFT BORDER AND RIGHT BORDER) OR (LB AND RB))

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5         38 DUP REM L4 (35 DUPLICATES REMOVED)

=> s l5 and trf
L6          0 L5 AND TRF

=> s promoter (3a) (CaMV or pea plastocyanin or high molecular weight glutenin or cassava mosaic
virus or commelina yellow mosaic virus)
L7       2137 PROMOTER (3A) (CAMV OR PEA PLASTOCYANIN OR HIGH MOLECULAR
WEIGHT
             GLUTENIN OR CASSAVA MOSAIC VIRUS OR COMMELINA YELLOW MOSAIC
             VIRUS)

=> s l5 and l7
L8          0 L5 AND L7

=> s l1 and l7
L9          0 L1 AND L7

=> d his

    (FILE 'HOME' ENTERED AT 15:51:26 ON 26 FEB 2003)

    FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 15:53:45 ON 26 FEB 2003
L1        351 S TRFA
L2       5371 S T-DNA
L3          1 S L1 AND L2
L4         73 S L2 AND ((LEFT BORDER AND RIGHT BORDER) OR (LB AND RB))
L5         38 DUP REM L4 (35 DUPLICATES REMOVED)
L6          0 S L5 AND TRF
L7       2137 S PROMOTER (3A) (CAMV OR PEA PLASTOCYANIN OR HIGH MOLECULAR WEI
L8          0 S L5 AND L7
L9          0 S L1 AND L7

=> d bib abs l5 1-10

L5  ANSWER 1 OF 38  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
    1
AN  2003:101627  BIOSIS
DN  PREV200300101627
TI  ***T*** - ***DNA***  insertional mutagenesis for activation tagging
    in rice.
AU  Jeong, Dong-Hoon; An, Suyoung; Kang, Hong-Gyu; Moon, Sunok; Han, Jong-Jin;
    Park, Sunhee; Lee, Hyun Sook; An, Kyungsook; An, Gynheung (1)
CS  (1) Department of Life Science and National Research Laboratory of Plant
    Functional Genomics, Pohang University of Science and Technology, Pohang,
    790-784, South Korea: genean@postech.ac.kr South Korea
SO  Plant Physiology (Rockville), (December 2002, 2002) Vol. 130, No. 4, pp.
    1636-1644. print.
    ISSN: 0032-0889.
```

DT Article
LA English
AB We have developed a new ***T*** - ***DNA*** vector, pGA2715, which can be used for promoter trapping and activation tagging of rice (Oryza sativa) genes. The binary vector contains the promoterless beta-glucuronidase (GUS) reporter gene next to the ***right*** ***border*** . In addition, the multimerized transcriptional enhancers from the cauliflower mosaic virus 35S promoter are located next to the ***left*** ***border*** . A total of 13,450 ***T*** - ***DNA*** insertional lines have been generated using pGA2715. Histochemical GUS assays have revealed that the GUS-staining frequency from those lines is about twice as high as that from lines transformed with the binary vector pGA2707, which lacks the enhancer element. This result suggests that the enhancer sequence present in the ***T*** - ***DNA*** improves the GUS-tagging efficiency. Reverse transcriptase-PCR analysis of a subset of randomly selected pGA2715 lines shows that expression of the genes immediately adjacent to the inserted enhancer is increased significantly. Therefore, the large population of ***T*** - ***DNA*** -tagged lines transformed with pGA2715 could be used to screen for promoter activity using the gus reporter, as well as for creating gain-of-function mutants.

L5 ANSWER 2 OF 38 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2002:549401 BIOSIS
DN PREV200200549401
TI Flanking sequence tags in Arabidopsis thaliana ***T*** - ***DNA*** insertion lines: A pilot study.
AU Ortega, Dominique; Raynal, Monique; Laudie, Michele; Llauro, Christel; Cooke, Richard; Devic, Martine; Genestier, Simone; Picard, Georges; Abad, Pierre; Contard, Pascale; Sarrobert, Catherine; Nussaume, Laurent; Bechtold, Nicole; Horlow, Christine; Pelletier, Georges; Delseny, Michel (1)
CS (1) Laboratoire "Genome et Developpement des Plantes", UMR 5096, CNRS-IRD-Universite de Perpignan, 52, Av. de Villeneuve, 66860, Perpignan Cedex: delseny@univ-perp.fr France
SO Comptes Rendus Biologies, (Juillet, 2002) Vol. 325, No. 7, pp. 773-780. http://www.elsevier.com/locate/issn/16310691. print.
ISSN: 1631-0691.
DT Article
LA English
AB Eight hundred and fifty Arabidopsis thaliana ***T*** - ***DNA*** insertion lines have been selected on a phenotypic basis. The ***T*** - ***DNA*** flanking sequences (FST) have been isolated using a PCR amplification procedure and sequenced. Seven hundred plant DNA sequences have been obtained revealing a ***T*** - ***DNA*** insertion in, or in the immediate vicinity of 482 annotated genes. Limited deletions of plant DNA have been observed at the site of insertion of ***T*** - ***DNA*** as well as in its left (***LB***) and right (***RB***) ***T*** - ***DNA*** signal sequences. The distribution of the ***T*** - ***DNA*** insertions along the chromosomes shows that they are essentially absent from the centrometric and pericentrometric regions.

L5 ANSWER 3 OF 38 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 2002:507585 BIOSIS
DN PREV200200507585
TI Transgene integration in aspen: Structures of integration sites and mechanism of ***T*** - ***DNA*** integration.
AU Kumar, Sandeep (1); Fladung, Matthias
CS (1) BFH, Institute for Forest Genetics and Forest Tree Breeding, Sieker Land Str. 2, 22927, Grosshansdorf: kumar@holz.uni-hamburg.de Germany
SO Plant Journal, (August, 2002) Vol. 31, No. 4, pp. 543-551. http://www.blackwell-science.com/ cgilib/jnlpage.bin?Journal=TPJ&File=TPJ&Page=aims. print.
ISSN: 0960-7412.
DT Article
LA English
AB To obtain insight into the mechanism of transferred DNA (***T*** - ***DNA***) integration in a long-lived tree system, we analysed 30 transgenic aspen lines. In total, 27 right ***T*** - ***DNA*** /plant junctions, 20 left ***T*** - ***DNA*** /plant junctions, and 10 target insertions from control plants were obtained. At the right end, the ***T*** - ***DNA*** was conserved up to the cleavage site in 18 transgenic lines (67%), and the ***right*** ***border*** repeat was deleted in nine junctions. Nucleotides from the ***left*** ***border*** repeat were present in 19 transgenic lines out of 20 cases analysed. However, only four (20%) of the ***left*** ***border*** ends were conserved to the processing end, indicating that the ***T*** - ***DNA*** left and right ends are treated mechanistically differently during the ***T*** - ***DNA*** integration process. Comparison of the genomic target sites prior to integration to the ***T*** - ***DNA*** revealed that the ***T*** - ***DNA*** inserted into the plant genome without any notable deletion of genomic sequence in three out of 10 transgenic lines analysed. However, deletions of DNA ranging in length from a few nucleotides to more than 500 bp were observed in other transgenic lines. Filler DNAs of up to 235 bp were observed on left and/or right junctions of six transgenic lines, which in most cases originated from the nearby host genomic sequence or from the ***T*** - ***DNA*** . Short sequence similarities between recombining strands near break points, in particular for the left ***T*** - ***DNA*** end, were observed in most of the lines analysed. These results confirm the well-accepted ***T*** - ***DNA*** integration model based on single-stranded annealing followed by ligation of the ***right*** ***border*** which is preserved by the VirD2 protein. However, a second category of ***T*** - ***DNA*** integration was also identified in nine transgenic lines, in which the ***right*** ***border*** of the ***T*** - ***DNA*** was partly truncated. Such integration events are described via a model for the repair of genomic double-strand breaks in somatic plant cells based on synthesis-dependent strand-annealing. This report in a long-lived tree system provides major insight into the mechanism of transgene integration.

L5 ANSWER 4 OF 38 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2001:196882 BIOSIS
DN PREV200100196882
TI Generation of selectable marker-free transgenic rice using double ***right*** - ***border*** (DRB) binary vectors.
AU Lu, Hui-Juan; Zhou, Xue-Rong; Gong, Zhu-Xun; Upadhyaya, Narayana M. (1)
CS (1) CSIRO Plant Industry, Canberra, ACT, 2601: N.Upadhyaya@pi.csiro.au Australia
SO Australian Journal of Plant Physiology, (2001) Vol. 28, No. 3, pp. 241-248. print.
ISSN: 0310-7841.
DT Article
LA English
SL English
AB Currently employed transformation systems require selectable marker genes encoding antibiotic or herbicide resistance, along with the gene of interest (GOI), to select transformed cells from among a large population of untransformed cells. The continued presence of these selectable markers, especially in food crops such as rice (Oryza sativa L.), is of increasing public concern. Techniques based on DNA recombination and Agrobacterium-mediated co-transformation with two binary vectors in a single or two different Agrobacterium strains, or with super-binary vectors carrying two sets of ***T*** - ***DNA*** border sequences (twin ***T*** - ***DNA*** vectors), have been employed by researchers to produce selectable marker-free (SMF) transgenic progeny. We have developed a double ***right*** - ***border*** (DRB) binary vector carrying two copies of ***T*** - ***DNA*** ***right*** - ***border*** (RB) sequences flanking a selectable marker gene, followed by a GOI and one copy of the ***left*** ***border*** sequence. Two types of ***T*** - ***DNA*** inserts, one initiated from the first RB containing both the selectable gene and the GOI, and the other from the second RB containing only the GOI, were expected to be produced and integrated into the genome. In the subsequent generation, these inserts could segregate away from each other, allowing the selection of the progeny with only the GOI. We tested this vector using two selectable marker genes and successfully obtained progeny plants in which the second selectable marker gene segregated away from the first. Using the DRB binary vector system, we recovered SMF transgenic lines containing a rice ragged stunt virus (RRSV)-derived synthetic resistance gene in the rice cultivars Jarrah and Xiu Shui. Approximately 36-64% of the primary transformants of these cultivars yielded SMF progeny. Among SMF Jarrah transgenic progeny <50% of plants contained the RRSV transgene. Thus, we have developed an efficient vector for producing SMF plants that allows straightforward cloning of any GOIs in comparison with the published 'twin ***T*** - ***DNA*** ' vectors.

L5 ANSWER 5 OF 38 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 2001:102648 BIOSIS
DN PREV200100102648
TI ***T*** - ***DNA*** vector backbone sequences are frequently integrated into the genome of transgenic plants obtained by Agrobacterium-mediated transformation.
AU De Buck, Sylvie; De Wilde, Chris; Van Montagu, Marc; Depicker, Ann (1)
CS (1) Vakgroep Molecutaire Genetica, Departement Plantengenetica, Vlaams Interuniversitair Instituut voor Biotechnologie, Universiteit Gent, K.L. Ledeganckstraat 35, 9000, Gent Belgium
SO Molecular Breeding, (October, 2000) Vol. 6, No. 5, pp. 459-468. print.
ISSN: 1380-3743.
DT Article
LA English
SL English
AB Transgenic Arabidopsis and tobacco plants (125) derived from seven Agrobacterium-mediated transformation experiments were screened by polymerase chain reaction and DNA gel blot analysis for the presence of vector 'backbone' sequences. The percentage of plants with vector DNA not belonging to the ***T*** - ***DNA*** varied between 20% and 50%. Neither the plant species, the explant type used for transformation, the replicon type nor the selection seem to have a major influence on the frequency of vector transfer. Only the border repeat sequence context could have an effect because ***T*** - ***DNA*** vector junctions were found in more than 50% of the plants of three different transformation series in which T-DNAs with octopine borders without inner border regions were used. Strikingly, many transgenic plants contain vector backbone sequences linked to the left ***T*** - ***DNA*** border as well as vector junctions with the right ***T*** - ***DNA*** border. DNA gel blots indicate that in most of these plants the complete vector sequence is integrated. We assume that integration into the plant genome of complete vector backbone sequences could be the result of a conjugative transfer initiated at the ***right*** ***border*** and subsequent continued copying at the left and right borders, called read-through. This model would imply that the ***left*** ***border*** is not frequently recognized as an initiation site for DNA transfer and that the ***right*** ***border*** is not efficiently recognized as a termination site for DNA transfer.

L5 ANSWER 6 OF 38 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 1999:98539 BIOSIS
DN PREV199900098539
TI Gene stability in transgenic aspen (Populus): I. Flanking DNA sequences and ***T*** - ***DNA*** structure.
AU Fladung, M. (1)
CS (1) Fed. Res. Cent. For. and Forest Prod., Inst. Forest Genet. and Forest Tree Breeding, Sieker Landstr. 2, D-22927 Grosshansdorf Germany
SO Molecular and General Genetics, (Jan., 1999) Vol. 260, No. 6, pp. 574-581.
ISSN: 0026-8925.
DT Article
LA English
AB The stability of transgenes in the genome of transformed plants depends strongly on their correct physical integration into the host genome as well as on flanking target DNA sequences. For long-lived species like trees, however, no information is available so far concerning inactivation or loss of transgenes due to gene silencing or somatic genome rearrangement events. In this study, four independently transformed 35S-rolC transgenic hybrid aspen plants (Populus tremula L. X tremuloides Michx.), each harbouring one copy of the transgene, were investigated during continuous growth in the greenhouse. In one of these transgenic lines (Esch5:35S-rolC-1) individuals frequently show phenotypic reversions, while in the remaining three lines (Esch5:35S-rolC-3, -5, -16) the gene was essentially stable. Molecular analysis including PCR, Southern and Northern assays clearly showed that the transgene had been lost in the revertant tissue of the unstable line. Sequencing of ***T*** - ***DNA*** right and left borders, and flanking DNA regions, in all four transgenic aspen lines revealed no differences either in the type of flanking DNA (G-C to A-T ratio) or with respect to the presence of enhancers or MAR (matrix associated repeats)-like structures. Primers located within the left and right flanking regions in the three stable lines could be used to recover the target sites from the untransformed plants. This was not possible, however, with the unstable line, indicating that at least one flanking sequence does not derive from the plant target

DNA but is of unknown origin. PCR using other primer pairs, and inverse PCR analysis, revealed an additional truncated ***T*** - ***DNA*** copy of 1050 nucleotides adjacent to the ***left*** ***border*** of the complete copy in this line. Sequencing of this truncated ***T*** - ***DNA*** revealed that it represented an inverted copy of part of the right half of the original construct. This special feature would allow the inverted repeat to pair with ***right*** ***border*** sequences of the complete copy. This would explain the frequently observed reversion resulting in transgene loss as due to intrachromosomal base-pairing leading to double-stranded loops of single-stranded DNA during mitotic cell divisions.

L5 ANSWER 7 OF 38 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 2000:103696 BIOSIS
DN PREV200000103696
TI The DNA sequences of ***T*** - ***DNA*** junctions suggest that complex ***T*** - ***DNA*** loci are formed by a recombination process resembling ***T*** - ***DNA*** integration.
AU De Buck, Sylvie; Jacobs, Anni; Van Montagu, Marc; Depicker, Ann (1)
CS (1) Vakgroep Moleculaire Genetica, Departement Plantengenetica, Vlaams Interuniversitair voor Biotechnologie (VIB), Universiteit Gent, K.L. Ledeganckstraat 35, B-9000, Gent Belgium
SO Plant Journal, (Nov., 1999) Vol. 20, No. 3, pp. 295-304. ISSN: 0960-7412.
DT Article
LA English
SL English
AB After Agrobacterium-mediated plant transformation, multiple T-DNAs frequently integrate at the same position in the plant genome, resulting in the formation of inverted and direct repeats. Because these inverted repeats cannot be amplified and analyzed by PCR, Arabidopsis root cells were co-transformed with two different T-DNAs with distinct sequences adjacent to the ***T*** - ***DNA*** borders. Nine direct or inverted ***T*** - ***DNA*** border junctions were analyzed at the sequence level. Precise end-to-end fusions were found between two ***right*** ***border*** ends, whereas imprecise fusions and filler DNA were present in ***T*** - ***DNA*** linkages containing a ***left*** ***border*** end. The results suggest that end-to-end ligation of double-stranded T-DNAs occurs especially between right ***T*** - ***DNA*** ends and that illegitimate recombination on the basis of microhomology, deletions, repair activities and insertions of filler DNA is involved in the formation of ***left*** ***border*** ***T*** - ***DNA*** junctions. Therefore, a similar illegitimate recombination mechanism is proposed that is involved in the formation of complex ***T*** - ***DNA*** inserts as well as in the integration of the ***T*** - ***DNA*** in the plant genome.

L5 ANSWER 8 OF 38 CAPLUS COPYRIGHT 2003 ACS
AN 1999:112549 CAPLUS
DN 130:307269
TI Gene stability in transgenic aspen (Populus). I. Flanking DNA sequences and ***T*** - ***DNA*** structure
AU Fladung, M.
CS Federal Research Centre for Forestry and Forest Products, Institute for Forest Genetics and Forest Tree Breeding, Grosshansdorf, D-22927, Germany
SO Molecular and General Genetics (1998), 260(6), 574-581 CODEN: MGGEAE; ISSN: 0026-8925
PB Springer-Verlag
DT Journal
LA English
AB The stability of transgenes in the genome of transformed plants depends strongly on their correct phys. integration into the host genome as well as on flanking target DNA sequences. For long-lived species like trees, however, no information is available so far concerning inactivation or loss of transgenes due to gene silencing or somatic genome rearrangement events. In this study, four independently transformed 35S-rolC transgenic hybrid aspen plants (Populus tremula L. x tremuloides Michx.), each harboring one copy of the transgene, were investigated during continuous growth in the greenhouse. In one of these transgenic lines (Esch5:35S-rolC-#1) individuals frequently show phenotypic reversions, while in the remaining three lines (Esch5:35S-rolC-#3, -#5, -#16) the gene was essentially stable. Mol. anal. including PCR, Southern and Northern assays clearly showed that the transgene had been lost in the revertant tissue of the unstable line. Sequencing of ***T*** - ***DNA*** right and left borders, and flanking DNA regions, in all four transgenic aspen lines revealed no differences either in the type of flanking DNA (G-C to A-T ratio) or with respect to the presence of enhancers or MAR (matrix assocd. repeats)-like structures. Primers located within the left and right flanking regions in the three stable lines could be used to recover the target sites from the untransformed plants. This was not possible, however, with the unstable line, indicating that at least one flanking sequence does not derive from the plant target DNA but is of unknown origin. PCR using other primer pairs, and inverse PCR anal., revealed an addnl. truncated ***T*** - ***DNA*** copy of 1050 nucleotides adjacent to the ***left*** ***border*** of the complete copy in this line. Sequencing of this truncated ***T*** - ***DNA*** revealed that it represented an inverted copy of part of the right half of the original construct. This special feature would allow the inverted repeat to pair with ***right*** ***border*** sequences of the complete copy. This would explain the frequently obsd. reversion resulting in transgene loss as due to intrachromosomal base-pairing leading to double-stranded loops of single-stranded DNA during mitotic cell divisions.
RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 38 CAPLUS COPYRIGHT 2003 ACS
AN 1998:590235 CAPLUS
DN 129:298899
TI Genome structure of pTi-SAKURA. (III). Characteristics of ***T*** - ***DNA***
AU Ohta, Nobuyuki; Murata, Kenji; Suzuki, Katsunori; Hattori, Yoshiyuki; Katoh, Akira; Yoshida, Kazuo
CS Department of Biological Science, Faculty of Science, Hiroshima University, Higashi-Hiroshima, 739-8526, Japan
SO Nucleic Acids Symposium Series (1998), 39, 185-186 CODEN: NACSD8; ISSN: 0261-3166
PB Oxford University Press
DT Journal
LA English
AB In nopaline type Ti plasmids, more than half of ***T*** - ***DNA*** had been still uncharacterized. By the complete sequencing of a Japanese nopaline type Ti plasmid pTi-SAKURA, we characterized open reading frames (ORF) on ***T*** - ***DNA*** . We found a single ***T*** - ***DNA*** of which distance between ***Left*** ***border*** (***LB***) and ***Right*** ***border*** (***RB***) sequences is 26,118bp. Av. GC content of the ***T*** - ***DNA*** was 47.4% which is much lower than 56.0% of the plasmid DNA av. There was a small region with high GC% which is an insertion sequence(IS) with two ORFs inside. Besides, we estd. 14 ORFs which are expected to function in plant cells. All the ORFs except one were homologous with those in T-DNAs of Ti and Ri plasmids. All 6 ORFs in nopaline type plasmids were highly homologous more than 97% with the pTi-SAKURA's ORFs. There was no nopaline type counterparts of the other ORFs esp. on left half area. We discussed the above characteristics in relation to agrobacterium's host range.
RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 10 OF 38 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1997:368775 BIOSIS
DN PREV199799658708
TI Effect of marker gene location in ***T*** - ***DNA*** on gene transfer from Agrobacterium to plant cells.
AU Ozcan, Sebahattin
CS Ankara Univ., Ziraat Fak., Tarla Bitkileri Bolumu, Diskapi, Ankara Turkey
SO Turkish Journal of Botany, (1997) Vol. 21, No. 4, pp. 189-195. ISSN: 1300-008X.
DT Article
LA English
SL English; Turkish
AB The present paper describes whether the efficiency of gene transfer from Agrobacterium tumefaciens to plant cells is influenced by the location of npt-II marker gene in ***T*** - ***DNA*** region. Therefore, binary vectors pSCV-Ori1 and pSCV-Ori2 were constructed and used for tobacco leaf disc transformation. pSCV-Ori1 harboured the npt-II marker gene adjacent to ***left*** ***border*** but 2.3 kb away from the ***right*** ***border*** , whereas pSCV-Ori2 contained the npt-II gene placed next to ***right*** ***border*** . Transformation experiments showed that pSCV-Ori2 plasmid resulted in higher frequency of kanamycin resistant callus clusters and shoots than pSCVOri1. This result clearly indicates that when the marker gene is placed adjacent to ***right*** ***border*** , it is transferred to plant cells more efficiently. However, cloning the marker gene a further 2.3 kb away from the ***right*** ***border*** reduced the transformation frequency. Integration of the marker gene into the tobacco genome was confirmed by PCR.

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(FILE 'HOME' ENTERED AT 15:51:26 ON 26 FEB 2003)

FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 15:53:45 ON 26 FEB 2003
L1 351 S TRFA
L2 5371 S T-DNA
L3 1 S L1 AND L2
L4 73 S L2 AND ((LEFT BORDER AND RIGHT BORDER) OR (LB AND RB))
L5 38 DUP REM L4 (35 DUPLICATES REMOVED)
L6 0 S L5 AND TRF
L7 2137 S PROMOTER (3A) (CAMV OR PEA PLASTOCYANIN OR HIGH MOLECULAR WEI
L8 0 S L5 AND L7
L9 0 S L1 AND L7

=> s l5 and binary
L10 11 L5 AND BINARY

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 11 ANSWERS - CONTINUE? Y/(N):y

L10 ANSWER 1 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2003:101627 BIOSIS
DN PREV200300101627
TI ***T*** - ***DNA*** insertional mutagenesis for activation tagging in rice.
AU Jeong, Dong-Hoon; An, Suyoung; Kang, Hong-Gyu; Moon, Sunok; Han, Jong-Jin; Park, Sunhee; Lee, Hyun Sook; An, Kyungsook; An, Gynheung (1)
CS (1) Department of Life Science and National Research Laboratory of Plant Functional Genomics, Pohang University of Science and Technology, Pohang, 790-784, South Korea: genean@postech.ac.kr South Korea
SO Plant Physiology (Rockville), (December 2002, 2002) Vol. 130, No. 4, pp. 1836-1844. print. ISSN: 0032-0889.
DT Article
LA English
AB We have developed a new ***T*** - ***DNA*** vector, pGA2715, which can be used for promoter trapping and activation tagging of rice (Oryza sativa) genes. The ***binary*** vector contains the promoterless beta-glucuronidase (GUS) reporter gene next to the ***right*** ***border*** . In addition, the multimerized transcriptional enhancers from the cauliflower mosaic virus 35S promoter are located next to the ***left*** ***border*** . A total of 13,450 ***T*** - ***DNA*** insertional lines have been generated using pGA2715. Histochemical GUS assays have revealed that the GUS-staining frequency from those lines is about twice as high as that from lines transformed with the ***binary*** vector pGA2707, which lacks the enhancer element. This result suggests that the enhancer sequence present in the ***T*** - ***DNA*** improves the GUS-tagging efficiency. Reverse transcriptase-PCR analysis of a subset of randomly selected pGA2715 lines shows that expression of the genes immediately adjacent to the inserted enhancer is increased significantly. Therefore, the large population of ***T*** - ***DNA*** -tagged lines transformed with pGA2715 could be used to screen for promoter activity using the gus reporter, as well as for creating gain-of-function mutants.

L10 ANSWER 2 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:196882 BIOSIS
DN PREV200100196882
TI Generation of selectable marker-free transgenic rice using double ***right*** - ***border*** (DRB) ***binary*** vectors.
AU Lu, Hui-Juan; Zhou, Xue-Rong; Gong, Zhu-Xun; Upadhyaya, Narayana M. (1)
CS (1) CSIRO Plant Industry, Canberra, ACT, 2601: N.Upadhyaya@pi.csiro.au Australia

SO Australian Journal of Plant Physiology, (2001) Vol. 28, No. 3, pp. 241-248. print.
ISSN: 0310-7841.
DT Article
LA English
SL English
AB Currently employed transformation systems require selectable marker genes encoding antibiotic or herbicide resistance, along with the gene of interest (GOI), to select transformed cells from among a large population of untransformed cells. The continued presence of these selectable markers, especially in food crops such as rice (Oryza sativa L.), is of increasing public concern. Techniques based on DNA recombination and Agrobacterium-mediated co-transformation with two ***binary*** vectors in a single or two different Agrobacterium strains, or with super-***binary*** vectors carrying two sets of ***T*** - ***DNA*** border sequences (twin ***T*** - ***DNA*** vectors), have been employed by researchers to produce selectable marker-free (SMF) transgenic progeny. We have developed a double ***right*** - ***border*** (DRB) ***binary*** vector carrying two copies of ***T*** - ***DNA*** ***right*** - ***border*** (RB) sequences flanking a selectable marker gene, followed by a GOI and one copy of the ***left*** ***border*** sequence. Two types of ***T*** - ***DNA*** inserts, one initiated from the first RB containing both the selectable gene and the GOI, and the other from the second RB containing only the GOI, were expected to be produced and integrated into the genome. In the subsequent generation, these inserts could segregate away from each other, allowing the selection of the progeny with only the GOI. We tested this vector using two selectable marker genes and successfully obtained progeny plants in which the second selectable marker gene segregated away from the first. Using the DRB ***binary*** vector system, we recovered SMF transgenic lines containing a rice ragged stunt virus (RRSV)-derived synthetic resistance gene in the rice cultivars Jarrah and Xiu Shui. Approximately 36-64% of the primary transformants of these cultivars yielded SMF progeny. Among SMF Jarrah transgenic progeny <50% of plants contained the RRSV transgene. Thus, we have developed an efficient vector for producing SMF plants that allows straightforward cloning of any GOIs in comparison with the published twin ***T*** - ***DNA*** ' vectors.

L10 ANSWER 3 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1997:366775 BIOSIS
DN PREV199799658708
TI Effect of marker gene location in ***T*** - ***DNA*** on gene transfer from Agrobacterium to plant cells.
AU Ozcan, Sebahattin
CS Ankara Univ., Ziraat Fak., Tarla Bitkileri Bolumu, Diskapi, Ankara Turkey
SO Turkish Journal of Botany, (1997) Vol. 21, No. 4, pp. 189-195.
ISSN: 1300-008X.
DT Article
LA English
SL English; Turkish
AB The present paper describes whether the efficiency of gene transfer from Agrobacterium tumefaciens to plant cells is influenced by the location of npt-II marker gene in ***T*** - ***DNA*** region. Therefore, ***binary*** vectors pSCV-Ori1 and pSCV-Ori2 were constructed and used for tobacco leaf disc transformation. pSCV-Ori1 harboured the npt-II marker gene adjacent to ***left*** ***border*** but 2.3 kb away from the ***right*** ***border*** , whereas pSCV-Ori2 contained the npt-II gene placed next to ***right*** ***border*** . Transformation experiments showed that pSCV-Ori2 plasmid resulted in higher frequency of kanamycin resistant callus clusters and shoots than pSCVOri1. This result clearly indicates that when the marker gene is placed adjacent to ***right*** ***border*** , it is transferred to plant cells more efficiently. However, cloning the marker gene a further 2.3 kb away from the ***right*** ***border*** reduced the transformation frequency. Integration of the marker gene into the tobacco genome was confirmed by PCR.

L10 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1997:110687 BIOSIS
DN PREV199799409870
TI Details of ***T*** - ***DNA*** structural organization from a transgenic Petunia population exhibiting co-suppression.
AU Cluster, Paul D.; O'Dell, Michael; Metzlaff, Michael; Flavell, Richard B. (1)
CS (1) Johns Innes Centre, Norwich Res. Park, Colney Lane, Norwich NR4 7UH UK
SO Plant Molecular Biology, (1996) Vol. 32, No. 6, pp. 1197-1203.
ISSN: 0167-4412.
DT Article
LA English
AB Analysis of Agrobacterium-transferred DNA (***T*** - ***DNA***) revealed strong correlations between transgene structures and floral pigmentation patterns from chalcone synthase (chs) co-suppression among 47 Petunia transformants. Presented here are the full details of ***T*** - ***DNA*** structural organization in that population. Sixteen transformants (34%) carried one ***T*** - ***DNA*** copy while 31 (66%) carried 106 complete and partial ***T*** - ***DNA*** elements in 54 linkage groups. Thirty linkage groups contained multiple ***T*** - ***DNA*** copies; 15 of these contained only contiguously repeated copies, 8 contained only dispersed copies and 7 contained both. ***Right*** - ***border*** inverted repeats were three times more frequent than ***left*** - ***border*** inverted or direct repeats. Large fragments of ***binary*** -vector sequences were linked to the ***T*** - ***DNA*** in seven plants.

L10 ANSWER 5 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:484709 BIOSIS
DN PREV199699199965
TI Deviating ***T*** - ***DNA*** transfer from Agrobacterium tumefaciens to plants.
AU Van Der Graaff, Eric; Den Dulk-Ras, Amke; Hooykaas, Paul J. J.
CS Inst. Mol. Plant Sci., Leiden Univ., Clusius Lab., Wassenaarseweg 64, 2333 AL Leiden Netherlands
SO Plant Molecular Biology, (1996) Vol. 31, No. 3, pp. 677-681.
ISSN: 0167-4412.
DT Article
LA English
AB We analyzed 29 ***T*** - ***DNA*** inserts in transgenic Arabidopsis thaliana plants for the junction of the ***right*** ***border*** sequences and the flanking plant DNA. DNA sequencing showed that in most lines the ***right*** ***border*** sequences transferred had been preserved during integration, corroborating literature data. Surprisingly, in four independent transgenic lines a complete ***right*** ***border*** repeat was present followed by ***binary*** vector sequences. Cloning of two of these ***T*** - ***DNA*** inserts by plasmid rescue showed that in these lines the transferred DNA consisted of the complete ***binary*** vector sequences in addition to the T-region. On the basis of the structure of the transferred DNA we propose that in these lines ***T*** - ***DNA*** transfer started at the ***left*** - ***border*** repeat, continued through the vector part, passed the ***right*** ***border*** repeat, and ended only after reaching again this ***left*** - ***border*** repeat.

L10 ANSWER 6 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:331947 BIOSIS
DN PREV199699054303
TI Analysis of octopine ***left*** ***border*** -directed DNA transfer from Agrobacterium to plants.
AU Ramanathan, Vai; Veluthambi, K. (1)
CS (1) Dep. Plant Biotechnol., Sch. Biotechnol., Madurai Kamaraj Univ., Madurai 625 021 India
SO Journal of Biosciences (Bangalore), (1996) Vol. 21, No. 1, pp. 45-56.
ISSN: 0250-5991.
DT Article
LA English
AB We constructed a ***binary*** plasmid, pVR30, with a neomycin phosphotransferase II (nptII) plant expression cassette flanked by a pTiA6 ***left*** ***border*** on its right and a pTiA6 ***right*** ***border*** on its left. This plasmid was used to study transfer of DNA to plants from a ***left*** ***border*** in the presence of a ***right*** ***border*** . Infection of tobacco leaf discs with a wild type octopine strain of Agrobacterium tumefaciens harbouring the ***binary*** plasmid resulted in the generation of kanamycin resistant calli at 18 to 26% frequency. Southern hybridization analysis of DNA isolated from eight transformed lines to different probes indicated that ***left*** ***border*** could mediate DNA transfer to plants in the presence of a ***right*** ***border*** in cis. Our results also suggest that transfer events corresponding to transfer of T-centre DNA of octopine Ti plasmid pTiA6 do occur. We have shown the relevance of ***left*** ***border*** -initiated ***T*** - ***DNA*** transfer by specifically selecting for such events and have confirmed it by Southern hybridization analysis. We also found that a border could be skipped in a few ***T*** - ***DNA*** transfer events.

L10 ANSWER 7 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1994:545973 BIOSIS
DN PREV199598005521
TI The small, versatile pPZP family of Agrobacterium ***binary*** vectors for plant transformation.
AU Hajdukiewicz, Peter; Svab, Zora; Maliga, Pal (1)
CS (1) Waksman Inst., Rutgers The State Univ. N.J., Piscataway, NJ 08855 USA
SO Plant Molecular Biology, (1994) Vol. 25, No. 6, pp. 989-994.
ISSN: 0167-4412.
DT Article
LA English
AB The new pPZP Agrobacterium ***binary*** vectors are versatile, relatively small, stable and are fully sequenced. The vectors utilize the pTiT37 ***T*** - ***DNA*** border regions, the pBR322 bom site for mobilization from Escherichia coli to Agrobacterium, and the ColE1 and pVS1 plasmid origins for replication in E. coli and in Agrobacterium, respectively. Bacterial marker genes in the vectors confer resistance to chloramphenicol (pPZP100 series) or spectinomycin (pPZP200 series), allowing their use in Agrobacterium strains with different drug resistance markers. Plant marker genes in the ***binary*** vectors confer resistance to kanamycin or to gentamycin, and are adjacent to the ***left*** ***border*** (***LB***) of the transferred region. A lacZ alpha-peptide, with the pUC18 multiple cloning site (MCS), lies between the plant marker gene and the ***right*** ***border*** (***RB***). Since the ***RB*** is transferred first, drug resistance is obtained only if the passenger gene is present in the transgenic plants.

L10 ANSWER 8 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1993:117903 BIOSIS
DN PREV199395062003
TI A versatile ***binary*** vector system with a ***T*** - ***DNA*** organisational structure conducive to efficient integration of cloned DNA into the plant genome.
AU Gleave, Andrew P.
CS Mol. Genetics Group, Plant Improvement Div., Hortic. Food Res. Inst. New Zealand Ltd., Private Bag 92021, Auckland New Zealand
SO Plant Molecular Biology, (1992) Vol. 20, No. 6, pp. 1203-1207.
ISSN: 0167-4412.
DT Article
LA English
AB A versatile gene expression cartridge and ***binary*** vector system was constructed for use in Agrobacterium-mediated plant transformation. The expression cartridge of the primary cloning vector, pART7, comprises of cauliflower mosaic virus Cabb B-JI isolate 35S promoter, a multiple cloning site and the transcriptional termination region of the octopine synthase gene. The entire cartridge can be removed from pART7 as a Not I fragment and introduced directly into the ***binary*** vector, pART27, recombinants being selected by blue/white screening for beta-galactosidase. pART27 carries the RK2 minimal replicon for maintenance in Agrobacterium, the ColE1 origin of replication for high-copy maintenance in Escherichia coli and the Tn7 spectinomycin/streptomycin resistance gene as a bacterial selectable marker. The organisational structure of the ***T*** - ***DNA*** of pART27 has been constructed taking into account the right to ***left*** ***border*** , 5' to 3' model of ***T*** - ***DNA*** transfer. The ***T*** - ***DNA*** carries the chimaeric kanamycin resistance gene (nopaline synthase promoter-neomycin phosphotransferase-nopaline synthase terminator) distal to the ***right*** ***border*** relative to the lacZ' region. Utilisation of these vectors in Agrobacterium-mediated transformation of tobacco demonstrated efficient ***T*** - ***DNA*** transfer to the plant genome.

L10 ANSWER 9 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1990:239430 BIOSIS
DN BA89:126383
TI IMPROVED ***BINARY*** VECTORS FOR AGROBACTERIUM MEDIATED PLANT TRANSFORMATION.
AU MCBRIDE K E; SUMMERFELT K R
CS CALGENE INC., 1920 FIFTH ST., DAVIS, CALIF. 95616, USA.
SO PLANT MOL BIOL, (1990) 14 (2), 269-276.

CODEN: PMBIDB. ISSN: 0167-4412.
FS BA; OLD
LA English
AB Improved plant transformation vectors were constructed which utilize the pRiHRI origin of replication for highly stable maintenance in Agrobacterium tumefaciens, the ColE1 origin of replication for high copy maintenance in Escherichia coli, and a gentamycin resistance gene as a strong selectable marker for bacteria. Concise ***T*** - ***DNA*** elements were engineered with border sequences from the TL-DNA of pTiA6, the Tn5 neomycin phosphotransferase gene (npt II) expressed from either CaMV 35S or mannopine synthase (mas) promoters, and the lac Z' gene segment from pUC18 as a source of unique restriction sites as well as an insertional inactivation marker for cloned DNA. The order of ***T*** - ***DNA*** components in all vectors is ***left*** ***border*** , plant marker cassette, lac Z', and ***right*** ***border*** , respectively. The prototype vector, pCGN1547, was shown to be very stable in A. tumefaciens strain LBA4404 and to act as an efficient donor of ***T*** - ***DNA*** in tomato transformation experiments. Use of the other vectors is also described.

L10 ANSWER 10 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1988:279348 BIOSIS
DN BA82:23211
TI ACTIVITY OF ***T*** ***DNA*** BORDERS IN PLANT CELL TRANSFORMATION BY MINI-T PLASMIDS.
AU JEN G C; CHILTON M-D
CS CIBA-GEIGY BIOTECHNOL. FAC., RESEARCH TRIANGLE PARK, NC 27709.
SO J BACTERIOL, (1988) 166 (2), 491-499.
CODEN: JOBAAY. ISSN: 0021-9193.
FS BA; OLD
LA English
AB By using a ***binary*** vector system, we examined the requirements for border sequences in ***T*** - ***DNA*** transformation of plant genomes. Mini-T plasmids consisting of small replicons with different extents of pTiT37 ***T*** - ***DNA*** were tested for plant tumor-inducing ability in Agrobacterium tumefaciens strain LBA4404 containing helper plasmid pAL4404 (which encodes virulence genes needed for ***T*** - ***DNA*** transfer). Assays of these bacteria on carrot disks, Kalanchoe leaves, and SR1 Nicotiana tabacum plantlets showed that mini-T plasmid containing full length ***T*** - ***DNA*** including left and right borders was highly virulent, as were mini-T plasmids containing all onc (oncogenicity) genes and only the ***right*** ***border*** . In contrast, mini-T plasmids containing all onc genes and only the ***left*** ***border*** induced tumors only rarely, and a mini-T plasmid containing all onc genes but no ***T*** - ***DNA*** borders was completely avirulent. Southern hybridization analyses of tumor DNA showed that ***T*** - ***DNA*** border sequences delimited the extent of the two-border mini-T plasmid transferred and integrated into the plant genome. When only one ***T*** - ***DNA*** border was present, it formed one end of the transferred DNA, and the other end mapped in the vector sequences. The implications of these results for the mechanism of ***T*** - ***DNA*** transfer and integration are discussed.

L10 ANSWER 11 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1986:243015 BIOSIS
DN BA82:7519
TI GENETIC STUDIES ON THE ROLE OF OCTOPINE ***T*** ***DNA*** BORDER REGIONS IN CROWN GALL TUMOR FORMATION.
AU RUBIN R A
CS BIOTECHNICA INTERNATIONAL INC., 85 BOLTON ST., CAMBRIDGE, MA 02140, USA.
SO MOL GEN GENET, (1986) 202 (2), 312-320.
CODEN: MGGEAE. ISSN: 0026-8925.
FS BA; OLD
LA English
AB Crown gall tumors result from transfer and integration of the ***T*** - ***DNA*** from the Ti plasmid of Agrobacterium tumefaciens into plant nuclear DNA. In the present study, recombinant plasmids containing deletion and rearrangement derivatives of the ***T*** - ***DNA*** region of the octopine Ti plasmid pTiA6 were tested in a ***binary*** tumorigenesis system (Hoekema et al. 1983) to determine the requirements for ***T*** - ***DNA*** border regions in tumor formation. Since two defined segments of the ***T*** - ***DNA*** region of octopine Ti plasmids can be detected in tumor DNA (the left (TL-) and right (TR-) DNA), four border regions exist in this Ti plasmid. Agrobacteria harboring plasmid constructs which contain a ***T*** - ***DNA*** gene capable of inciting tumors (gene 4, the tmr gene, which is involved in cytokinin biosynthesis) and various ***T*** - ***DNA*** border regions were tested for ability to cause tumors on Nicotiana glauca and other host plants. Such tmr constructs containing as their only border region the ***right*** ***border*** of either the TL-DNA or the TR-DNA are fully tumorigenic. Analogous tmr constructs containing only the TL-DNA ***left*** ***border*** region are not tumorigenic. These results do not depend on the orientation or position of the single border with respect to the tmr gene; furthermore, the TR-DNA ***right*** ***border*** can confer tumor-forming ability despite the presence of an intervening copy of the TL-DNA ***left*** ***border*** . These results for relatively small plasmids are contrasted with previously determined requirements for border regions in tumorigenesis by intact Ti plasmids. A model previously proposed by Wang et al. (1984) for the role of border regions in DNA transfer to plant cells is extended in order to explain the tumor-forming ability of plasmid constructs containing a single border region. The results of this study interpreted according to the model suggest that the octopine TL-DNA ***left*** ***border*** is defective in this DNA-transfer process.

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FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 15:53:45 ON 26 FEB 2003
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L2 5371 S T-DNA
L3 1 S L1 AND L2
L4 73 S L2 AND ((LEFT BORDER AND RIGHT BORDER) OR (LB AND RB))
L5 38 DUP REM L4 (35 DUPLICATES REMOVED)
L6 0 S L5 AND TRF
L7 2137 S PROMOTER (3A) (CAMV OR PEA PLASTOCYANIN OR HIGH MOLECULAR WEI
L8 0 S L5 AND L7
L9 0 S L1 AND L7
L10 11 S L5 AND BINARY

=> s promoter (5a) (CaMV or pea plastocyanin or high molecular weight glutenin or cassava mosaic virus or commelina yellow mosaic virus)
L11 2271 PROMOTER (5A) (CAMV OR PEA PLASTOCYANIN OR HIGH MOLECULAR WEIGHT GLUTENIN OR CASSAVA MOSAIC VIRUS OR COMMELINA YELLOW MOSAIC VIRUS)

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L12 0 L11 AND L1

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L13 2 L1 AND REVIEW

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L14 2 DUP REM L13 (0 DUPLICATES REMOVED)

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L14 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS
AN 1999:28350 CAPLUS
DN 130:232884
TI ***TrfA*** dimers play a role in copy-number control of RK2 replication
AU Toukdarian, Aresa E.; Helinski, Donald R.
CS Center for Molecular Genetics and Department of Biology, University of California, San Diego, La Jolla, CA, 92093-0322, USA
SO Gene (1998), 223(1-2), 205-211
CODEN: GENED6; ISSN: 0378-1119
PB Elsevier Science B.V.
DT Journal; General Review
LA English
AB A ***review*** with 16 refs. Copy-no. regulation of the broad-host-range plasmid RK2 is dependent on the plasmid-encoded initiator protein, ***TrfA*** , and the RK2 origin of replication. The handcuffing model for copy-no. control proposes that ***TrfA*** -bound oris reversibly couple to prevent the further initiation of plasmid replication when the copy no. in vivo is at or above the replicon-specific copy no. ***TrfA*** mutants have been isolated which allow for oriV replication at elevated copy nos. To better understand the mechanism of 'handcuffing', the copy-up ***TrfA*** (G254D/S267L) mutant was characterized further. In the present study we show by size exclusion chromatog. and native gel electrophoresis that unlike wt ***TrfA*** which is largely dimeric, purified His6- ***TrfA*** (G254D/S267L) is primarily monomeric. In vivo, TrfA33(G254D/S267L) supports replication of an RK2 ori plasmid in trans at a greatly elevated copy no., while in cis the plasmid exhibits runaway replication. However, expression of either of two previously isolated DNA-binding defective ***TrfA*** mutants, TrfA33(P151S) or TrfA33(S257F), in a cell transformed with a mini-RK2 replicon encoding TrfA33(G254D/S267L) results in suppression of the runaway phenotype. His6- ***TrfA*** (P151S) and His6- ***TrfA*** (S257F) purify as dimers, and when expressed in vivo are incapable of supporting RK2 plasmid replication. In contrast, combination of the ***trfA*** (P151S) or ***trfA*** (S257F) mutation with the ***trfA*** (G254D/S267L) mutations results in the expression of mutant ***TrfA*** proteins which are mainly monomers and which can no longer restore copy control to replication directed by TrfA33(G254D/S267L) in vivo. On the basis of these findings a handcuffing model is proposed, whereby oriV-bound ***TrfA*** monomers are coupled by dimeric ***TrfA*** mols.
RE.CNT 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

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L14 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS
AN 1994:526158 CAPLUS
DN 121:126158
TI Complete nucleotide sequence of Birmingham IncP.alpha. plasmids. Compilation and comparative analysis
AU Pansegrau, Werner; Lanka, Erich; Barth, Peter T.; Figurski, David H.; Guiney, Donald G.; Haas, Dieter; Helinski, Donald R.; Schwab, Helmut; Stanisich, Vilma A.; Thomas, Christopher M.
CS Max-Planck-Inst. Mol. Genet., Berlin, D-14195, Germany
SO Journal of Molecular Biology (1994), 239(5), 623-63
CODEN: JMOBAK; ISSN: 0022-2836
DT Journal; General Review
LA English
AB A ***review*** with over 100 refs. The IncP.alpha. promiscuous plasmid (R18, R68, RK2, RP1 and RP4) comprises 60,099 bp of nucleotide sequence, encoding at least 74 genes. About 40 kb of the genome, designated the IncP core and including all essential replication and transfer functions, can be aligned with equiv. sequences in the IncP.beta. plasmid R751. The compiled IncP.alpha. sequence revealed several previously unidentified reading frames that are potential genes. IncP.alpha. plasmids carry genetic information very efficiently: the coding sequences of the genes are closely packed but rarely overlap, and occupy almost 86% of the genome's nucleotide sequence. All of the 74 genes should be expressed, although there is as yet exptl. evidence for expression of only 60 of them. Six examples of tandem-in-frame initiation sites specifying two gene products each are known. Two overlapping gene arrangements occupy different reading frames of the same region. Intergenic regions include most of the 25 promoters; transcripts are usually polycistronic. Translation of most of the open reading frames seems to be initiated independently, each from its own ribosomal binding and initiation site, although, a few cases of coupled translation have been reported. The most frequently used initiation codon is AUG but translation for a few open reading frames begins at GUG or UUG. The most common stop-codon is UGA followed by UAA and then UAG. Regulatory circuits are complex and largely dependent on two components of the central control operon. KorA and KorB are transcriptional repressors controlling at least seven operons. KorA and KorB act synergistically in several cases by recognizing and binding to conserved nucleotide sequences. Twelve KorB binding sites were found around the IncP.alpha. sequence and these are conserved in R751 (IncP.beta.) with respect to both sequence and location. Replication of IncP.alpha. plasmids requires oriV and the plasmid-encoded initiator protein ***TrfA*** in combination with the host-encoded replication machinery. Conjugative plasmid transfer depends on two sep. regions occupying about half of the genome. The primary segregational stability system designated ParMrs consists of a

putative site-specific recombinase, a possible partitioning app. and a post-segregational lethality mechanism, all encoded in two divergent operons. Proteins related to the products of F sop and P1 par partitioning genes are sep. encoded in the central control operon.

=> d his'
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The following are valid formats:

ABS ——— GI and AB
ALL ——— BIB, AB, IND, RE
APPS ——— AI, PRAI
BIB ——— AN, plus Bibliographic Data and PI table (default)
CAN ——— List of CA abstract numbers without answer numbers
CBIB ——— AN, plus Compressed Bibliographic Data
DALL ——— ALL, delimited (end of each field identified)
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FBIB ——— AN, BIB, plus Patent FAM
IND ——— Indexing data
IPC ——— International Patent Classifications
MAX ——— ALL, plus Patent FAM, RE
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SAM ——— CC, SX, TI, ST, IT
SCAN ——— CC, SX, TI, ST, IT (random display, no answer numbers; SCAN must be entered on the same line as the DISPLAY, e.g., D SCAN or DISPLAY SCAN)
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HITSTR ——— HIT RN, its text modification, its CA index name, and its structure diagram
HITSEQ ——— HIT RN, its text modification, its CA index name, its structure diagram, plus NTE and SEQ fields
FHITSTR ——— First HIT RN, its text modification, its CA index name, and its structure diagram
FHITSEQ ——— First HIT RN, its text modification, its CA index name, its structure diagram, plus NTE and SEQ fields
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OCC ——— Number of occurrence of hit term and field in which it occurs

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L14 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS
AN 1999:28350 CAPLUS
DN 130:232884
TI ***TrfA*** dimers play a role in copy-number control of RK2 replication
AU Toukdarian, Aresa E.; Helinski, Donald R.
CS Center for Molecular Genetics and Department of Biology, University of California, San Diego, La Jolla, CA, 92093-0322, USA
SO Gene (1998), 223(1-2), 205-211
CODEN: GENED6; ISSN: 0378-1119
PB Elsevier Science B.V.
DT Journal; General Review
LA English
RE.CNT 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L1 351 S TRFA
L2 5371 S T-DNA
L3 1 S L1 AND L2
L4 73 S L2 AND ((LEFT BORDER AND RIGHT BORDER) OR (LB AND RB))
L5 38 DUP REM L4 (35 DUPLICATES REMOVED)
L6 0 S L5 AND TRF
L7 2137 S PROMOTER (3A) (CAMV OR PEA PLASTOCYANIN OR HIGH MOLECULAR WEI
L8 0 S L5 AND L7
L9 0 S L1 AND L7
L10 11 S L5 AND BINARY
L11 2271 S PROMOTER (5A) (CAMV OR PEA PLASTOCYANIN OR HIGH MOLECULAR WEI
L12 0 S L11 AND L1
L13 2 S L1 AND REVIEW
L14 2 DUP REM L13 (0 DUPLICATES REMOVED)

=> s rk2 and l1
L15 252 RK2 AND L1

=> s l1 and copy number
L16 42 L1 AND COPY NUMBER

=> dup rem l16
PROCESSING COMPLETED FOR L16
L17 21 DUP REM L16 (21 DUPLICATES REMOVED)

=> s l17 not l14
L18 21 L17 NOT L14

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 21 ANSWERS - CONTINUE? Y/(N):y

L18 ANSWER 1 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:582235 BIOSIS
DN PREV200200582235
TI Conditionally amplifiable BACs: Switching from single-copy to high-copy vectors and genomic clones.
AU Wild, Jadwiga; Hradecna, Zdenka; Szybalski, Waclaw (1)
CS (1) McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, WI, 53706: szybalski@oncology.wisc.edu USA
SO Genome Research, (September, 2002) Vol. 12, No. 9, pp. 1434-1444. http://www.genome.org/. print.
ISSN: 1088-9051.
DT Article
LA English
AB The widely used, very-low-copy BAC (bacterial artificial chromosome) vectors are the mainstay of present genomic research. The principal advantage of BACs is the high stability of inserted clones, but an important disadvantage is the low yield of DNA, both for vectors alone and when carrying genomic inserts. We describe here a novel class of single-copy/high-copy (SC/HC) pBAC/oriV vectors that retain all the advantages of low-copy BAC vectors, but are endowed with a conditional and tightly controlled oriV/ ***TrfA*** amplification system that allows: (1) a yield of apprx100 copies of the vector per host cell when conditionally induced with L-arabinose, and (2) analogous DNA amplification (only upon induction and with ***copy*** ***number*** depending on the insert size) of pBAC/oriV clones carrying >100-kb inserts. Amplifiable clones and libraries facilitate high-throughput DNA sequencing and other applications requiring HC plasmid DNA. To turn on DNA amplification, which is driven by the oriV origin of replication, we used copy-up mutations in the gene ***trfA*** whose expression was very tightly controlled by the araC-ParaBAD promoter/regulator system. This system is inducible by L-arabinose, and could be further regulated by glucose and fucose. Amplification of DNA upon induction with L-arabinose and its modulation by glucose are robust and reliable. Furthermore, we discovered that addition of 0.2% D-glucose to the growth medium helped toward the objective of obtaining a real SC state for all BAC systems, thus enhancing the stability of their maintenance, which became equivalent to cloning into the host chromosome.

L18 ANSWER 2 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:86917 BIOSIS
DN PREV199900086917
TI ***TrfA*** dimers play a role in ***copy*** - ***number*** control of RK2 replication.
AU Toukdarian, Aresa E.; Helinski, Donald R. (1)
CS (1) Dep. Biology, Univ. Calif., San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0322 USA
SO Gene (Amsterdam), (Nov. 26, 1998) Vol. 223, No. 1-2, pp. 205-211.
ISSN: 0378-1119.
DT Article
LA English
AB ***Copy*** - ***number*** regulation of the broad-host-range plasmid RK2 is dependent on the plasmid-encoded initiator protein, ***TrfA***, and the RK2 origin of replication. The handcuffing model for ***copy*** - ***number*** control proposes that ***TrfA*** -bound oris reversibly couple to prevent the further initiation of plasmid replication when the ***copy*** ***number*** in vivo is at or above the replicon-specific ***copy*** ***number***. ***TrfA*** mutants have been isolated which allow for oriV replication at elevated copy numbers. To better understand the mechanism of 'handcuffing', the copy-up ***TrfA*** (G254D/S267L) mutant was characterized further. In the present study we show by size exclusion chromatography and native gel electrophoresis that unlike wt ***TrfA*** which is largely dimeric, purified His6- ***TrfA*** (G254D/S267L) is primarily monomeric. In vivo, TrfA33(G254D/S267L) supports replication of an RK2 ori plasmid in trans at a greatly elevated ***copy*** ***number***, while in cis the plasmid exhibits runaway replication. However, expression of either of two previously isolated DNA-binding defective ***TrfA*** mutants, TrfA33(P151S) or TrfA33(S257F), in a cell transformed with a mini-RK2 replicon encoding TrfA33(G254D/S267L) results in suppression of the runaway phenotype. His6- ***TrfA*** (P151S) and His6- ***TrfA*** (S257F) purify as dimers, and when expressed in vivo are incapable of supporting RK2 plasmid replication. In contrast, combination of the ***trfA*** (P151S) or ***trfA*** (S257F) mutation with the ***trfA*** (G254D/S267L) mutations results in the expression of mutant ***TrfA*** proteins which are mainly monomers and which can no longer restore copy control to replication directed by TrfA33(G254D/S267L) in vivo. On the basis of these findings a handcuffing model is proposed, whereby oriv-bound ***TrfA*** monomers are coupled by dimeric ***TrfA*** molecules.

L18 ANSWER 3 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:86901 BIOSIS
DN PREV199900086901
TI Targeting and retrofitting pre-existing libraries of transposon insertions with FRT and oriV elements for in-vivo generation of large quantities of any genomic fragment.
AU Wild, Jadwiga; Sektas, Marian; Hradecna, Zdenka; Szybalski, Waclaw (1)
CS (1) McArdle Lab. Cancer Res., Univ. Wis. Med. Sch., 1400 University Avenue, Madison, WI 53706 USA
SO Gene (Amsterdam), (Nov. 26, 1998) Vol. 223, No. 1-2, pp. 55-66.
ISSN: 0378-1119.
DT Article
LA English
AB A procedure is described that converts the pre-existing transposon insertion libraries to a collection of 'pop-out' strains, each allowing generation of 20- to 100-kb genomic fragments directly from the genome. The procedure consists of two steps: (1) single transposon insertions are targeted and retrofitted with excision and amplification elements (FRT and oriV), by homologous recombination with an FRT-oriV-carrying plasmid; and (2) two retrofitted neighboring transposons are brought together by PI transduction. From each strain, a 20- to 100-kb genomic fragment, bound by

a pair of retrofitted transposons, could be excised and amplified upon supplying in trans the excision (Flp) and replication (***TrfA***) functions. To enhance the efficiency of crossing-in the FRT-ori V cassette, we transiently increased the ***copy*** ***number*** of our retrofitting plasmids using a temperature-sensitive ***TrfA*** -supplying helper plasmid. Using FRT-oriV and helper plasmids, we retrofitted four Tn10Km(R) and three Tn10Cm(R) insertions. Subsequently, the FRT-ori V retrofitted insertions were crossed with each other in pairs (Km(R) X Cm(R)), using P1 phage transductions. The resulting Cm(R)FRT-(28-65-kb)-KM(R)FRT strains were transformed with a plasmid expressing FLP and ***trfA*** genes from the tightly controlled Ptet promoter. Induction of this tightly repressed promoter by autoclaved chlortetracycline (cTc) resulted in the efficient excision and amplification of genomic fragments located between FRT sites, but only in productive strains, i.e. having two parallel FRTs. We have shown that genomic fragments of 28-, 40-, 50- and 65-kb were efficiently excised and amplified. Furthermore, we could convert non-productive strains (having FRTs in non-parallel orientation), to productive combination of parallel FRTs, because one of the FRT elements was flanked by two convergent loxP sites, and thus could be inverted by the Cre function delivered either by the P1 phage or by a specially constructed temperature-sensitive Ptac-cre plasmid. Although several microbial genomes were recently sequenced, the described method will help in supplying large quantities of any genomic fragment (prepared without the conventional cloning and its artifacts) for refined sequence comparison among strains and species, and for further analysis of uncharacterized ORFs, various mutations, and regulatory elements or functions. The excised and circularized DNA fragments (plasmids) could be propagated like any other large plasmids but only in hosts that could supply the appropriate Rep function. Our original 'pop-out' method (Posfai et al. (1994) Nucleic Acids Res. 22, 2392-2398) was already employed for sequencing of the E. coli genome (Blattner et al. (1997) Science 277, 1453-1474). Moreover, the Flp-mediated recombination between two FRT elements resulted in bacterial strains with large deletions (for parallel FRT orientations) or with large inversions (for inverted FRT orientations).

L18 ANSWER 4 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1998:495497 BIOSIS
DN PREV199800495497
TI Complete sequence of the IncPbeta plasmid R751: Implications for evolution and organisation of the IncP backbone.
AU Thorsted, Peter B.; Macartney, Donia P.; Akhtar, Parveen; Haines, Anthony S.; Ali, Nasima; Davidson, Philip; Stafford, Theresa; Pocklington, Michael J.; Pansegrau, Werner; Wilkins, Brian M.; Lanka, Erich; Thomas, Christopher M. (1)
CS (1) Sch. Biol. Sci., Univ. Birmingham, Edgbaston, Birmingham B15 2TT UK
SO Journal of Molecular Biology, (Oct. 9, 1998) Vol. 282, No. 5, pp. 969-990. ISSN: 0022-2836.
DT Article
LA English
AB The broad host range IncP plasmids are of particular interest because of their ability to promote gene spread between diverse bacterial species. To facilitate study of these plasmids we have compiled the complete sequence of the IncPbeta plasmid R751. Comparison with the sequence of the IncPalpha plasmids confirms the conservation of the IncP backbone of replication, conjugative transfer and stable inheritance functions between the two branches of this family. As in the IncPalpha genome the DNA of this backbone appears to have been enriched for the GCCG/CGGC motifs characteristic of the genome of organisms with a high G + C content, such as P. aeruginosa, suggesting that IncPbeta plasmids have been subjected during their evolution to similar mutational and selective forces as IncPalpha plasmids and may have evolved in pseudomonad hosts. The IncP genome is consistently interrupted by insertion of phenotypic markers and/or transposable elements between oriV and ***trfA*** and between the tra and trb operons. The R751 genome reveals a family of repeated sequences in these regions which may form the basis of a hot spot for insertion of revealed that it is not a member of the Tn21 family as we had proposed previously from an inspection of its ends. Rather it is a composite transposon defined by inverted repeats of a 1347 bp IS element belonging to a recently discovered family which is distributed throughout the prokaryotes. The central unique region of Tn4321 encodes two predicted proteins, one of which is a regulatory protein while the other is presumably responsible for an as yet unidentified phenotype. The most striking feature of the IncPalpha plasmids, the global regulation of replication and transfer by the KorA and KorB proteins encoded in the central control operon, is conserved between the two plasmids although there appear to be significant differences in the specificity of repressor-operator interactions. The importance of these global regulatory circuits is emphasised by the observation that the operator sequences for KorB are highly conserved even in contexts where the surrounding region, either a protein coding or intergenic sequence, has diverged considerably. There appears to be no equivalent of the parABCDE region which in the IncPa plasmids provides multimer resolution, lethality to plasmid-free segregants and active partitioning functions. However, we found that the continuous sector from co-ordinate 0 to 9100 bp, encoding the co-regulated kle and kle operons as well as the central control region, could confer a high degree of segregational stability on a low ***copy*** ***number*** test vector. Thus R751 appears to exhibit very clearly what was first revealed by study of the IncPalpha plasmids, namely a fully functional coordinately regulated set of replication, transfer and stable inheritance functions.

L18 ANSWER 5 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1997:453250 BIOSIS
DN PREV199799752453
TI Improved broad-host-range RK2 vectors useful for high and low regulated gene expression levels in Gram-negative bacteria.
AU Blatny, Janet Martha (1); Brautaset, Trygve; Winther-Larsen, Hanne C.; Karunakaran, Ponniah; Valla, Svein
CS (1) UNIGEN Cent. Mol. Biol., Norwegian Univ. Sch. Technol., N-7005 Trondheim Norway
SO Plasmid, (1997) Vol. 38, No. 1, pp. 35-51. ISSN: 0147-619X.
DT Article
LA English
AB This report describes the construction and use of improved broad-host-range expression vectors based on the previously constructed pJB137 and pJB653 plasmids (Blatny et al., 1997). These vectors contain the minimal replicon of RK2 and the inducible Pu or Pm promoters together with their regulatory xylR or xylS genes, respectively, from the Pseudomonas putida TOL plasmid pWW0. A set of ATG vectors were derived from pJB653, and these vectors are characterized by the relatively small size, the presence of multiple cloning sites downstream of Pm, the establishment of their nucleotide sequence, the presence of RK2 oriT, and different antibiotic selection markers. The copy numbers of all the vectors can easily be modified by using copy-up mutations of the ***trfA*** gene, required for initiation of replication of RK2 replicons. The vectors were used to study the expression levels of the Acetobacter xylinum phosphoglucomutase gene celB and the two commonly used reporter genes luc and cat in Escherichia coli, Pseudomonas aeruginosa, and Xanthomonas campestris. Good induction properties and tight regulation of Pm were achieved in all three species tested, and higher gene expression levels were obtained by using the ATG vectors compared to pJB653. By introducing different ***trfA*** copy-up mutations into the vectors, a wide range of gene expression levels from Pu and Pm were obtained in E. coli. Induced expression levels of luc, cat, and celB from Pm were found to be comparable to or higher than those from the Ptrc and P-T7 promoters located on high ***copy*** ***number*** plasmids. The induced levels of Luc activity were higher in P. aeruginosa than in E. coli, indicating that these vectors may be useful for maximization of gene expression in strains other than E. coli. We believe that the well-characterized vectors described here are useful for gene expression studies and routine cloning experiments in many Gram-negative bacteria.

L18 ANSWER 6 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:242646 BIOSIS
DN PREV199698790775
TI Copy-up mutants of the plasmid RK2 replication initiation protein are defective in coupling RK2 replication origins.
AU Blasina, Alessandra; Kittell, Barbara L.; Toukdarian, Aresa E.; Helinski, Donald R. (1)
CS (1) Dep. Biol., Univ. California San Diego, La Jolla, CA 92093-0634 USA
SO Proceedings of the National Academy of Sciences of the United States of America, (1996) Vol. 93, No. 8, pp. 3559-3564. ISSN: 0027-8424.
DT Article
LA English
AB The broad host range plasmid RK2 replicates and regulates its ***copy*** ***number*** in a wide range of Gram-negative bacteria. The plasmid-encoded trans-acting replication protein ***TrfA*** and the origin of replication oriV are sufficient for controlled replication of the plasmid in all Gram-negative bacteria tested. The ***TrfA*** protein binds specifically to direct repeat sequences (iterons) at the origin of replication. A replication control model, designated handcuffing or coupling, has been proposed whereby the formation of coupled ***TrfA*** -oriV complexes between plasmid molecules results in hindrance of origin activity and, consequently, a shut-down of plasmid replication under conditions of higher than normal ***copy*** ***number*** . Therefore, according to this model, the coupling activity of an initiation protein is essential for ***copy*** ***number*** control and a copy-up initiation protein mutant should have reduced ability to form coupled complexes. To test this model for plasmid RK2, two previously characterized copy-up ***TrfA*** mutations, ***trfA*** -254D and ***trfA*** -2671, were combined and the resulting copy-up double mutant ***TrfA*** protein ***TrfA*** -254D/267L was characterized. Despite initiating runaway (uncontrolled) replication in vivo, the copy-up double-mutant ***TrfA*** protein exhibited replication kinetics similar to the wild-type protein in vitro. Purified ***TrfA*** -254D, ***TrfA*** -267L, and ***TrfA*** -254D/267L proteins were then examined for binding to the iterons and for coupling activity using an in vitro ligase-catalyzed multimerization assay. It was found that both single and double ***TrfA*** mutant proteins exhibited substantially reduced (single mutants) or barely detectable (double mutant) levels of coupling activity while not being diminished in their capacity to bind to the origin of replication. These observations provide direct evidence in support of the coupling model of replication control.

L18 ANSWER 7 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:59752 BIOSIS
DN PREV199698831887
TI Dissection of the core and auxiliary sequences in the vegetative replication origin of promiscuous plasmid RK2.
AU Shah, Deepan S.; Cross, Michael A.; Porter, David; Thomas, Christopher M. (1)
CS (1) Sch. Biological Sci., Univ. Birmingham, Edgbaston, Birmingham B15 2TT UK
SO Journal of Molecular Biology, (1995) Vol. 254, No. 4, pp. 608-622. ISSN: 0022-2836.
DT Article
LA English
AB The vegetative replication origin (oriV) of promiscuous IncP plasmid RK2 can function in many Gram-negative bacterial species when supplied with the plasmid-encoded replication protein ***TrfA*** and host-encoded replication proteins including DnaA. Nine ***TrfA*** binding sites (iterons) are known, and also two DnaA binding sites, box 1, between ***TrfA*** iterons 4 and 5, and box 2, downstream of repeat 9. The deletion analysis presented here shows that the core oriV requires DnaA box 1 for function in Escherichia coli and Pseudomonas putida. This DnA box is not essential in Pseudomonas aeruginosa, although its deletion does reduce plasmid ***copy*** ***number*** in this species. A putative IHF binding site is located upstream of DnaA box 1, but IHF deficiency in E. coli seems not to alter replication efficiency or ***copy*** ***number*** control. Cloned oriV can interfere with maintenance of an independent RK2 replicon. Analysis of replication inhibition functions associated with oriV showed that a short putative orf between ***TrfA*** iterons 1 and 2 is not necessary for replication inhibition, the presence of repeats 5 to 9 in target and inhibitor plasmid are not sufficient for efficient inhibition and inhibition does not correlate directly with the number of direct repeats present. Rather, the results showed that the isolated repeats 1 and 2 to 4, potentiate replication inhibition disproportionately to their effect on the number of ***TrfA*** binding sites. The results are consistent with the idea that repeats 1 to 4, arranged as a single copy and as an irregular group of three, potentiate the ability of the oriV region to form complexes which inhibit replication. We suggest that ***TrfA*** bound at these iterons may be more susceptible to forming pairs between oriV sequences on different plasmids.

L18 ANSWER 8 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1995:171324 BIOSIS
DN PREV199598185624
TI The host range of RK2 minimal replicon copy-up mutants is limited by species-specific differences in the maximum tolerable ***copy*** ***number*** .

AU Haugan, Kare; Karunakaran, Ponniah; Tondervik, Anne; Valla, Svein (1)
CS (1) UNIGEN Cent. Mol. Biol., Univ. Trondheim, N-7005 Trondheim Norway
SO Plasmid, (1995) Vol. 33, No. 1, pp. 27-39.
ISSN: 0147-619X.
DT Article
LA English
AB The minimal replicon of the broad-host-range plasmid RK2 consists of a gene, ***trfA*** (trans-acting replication), encoding a protein required for initiation of plasmid replication. The ***TrfA*** protein binds to iterons in the cis-acting origin of vegetative replication (oriV), but the exact mechanism by which ***TrfA*** -mediated replication initiation takes place is not known. We report here the isolation and characterization of five mini RK2 ***trfA*** mutant plasmids with an elevated plasmid ***copy*** ***number*** , four in Pseudomonas aeruginosa and one in Azotobacter vinelandii. The mutations are localized between or downstream of previously reported Escherichia coli copy-up mutations in ***trfA*** , and one of the mutations has been described earlier as an independent copy-up isolate in E. coli. The five mutant plasmids were all moderately copy up in both E. coli and their host of origin, in spite of the use of isolation procedures which were expected to select efficiently in favor of plasmid mutants specifying high copy numbers. In contrast, previously described high copy-up mutants isolated in E. coli could not be established in P. aeruginosa and A. vinelandii. These high copy-up mutants were shown to induce cell killing in E. coli under conditions where the plasmid ***copy*** ***number*** was increased as a physiological response to reduced growth rate. We propose that the reason for this killing effect is that the ***copy*** ***number*** under these conditions exceeds an upper tolerance level specific for E. coli. By assuming that the corresponding tolerance level is lower in P. aeruginosa and A. vinelandii than in E. coli, and that the mechanism of ***copy*** ***number*** regulation is similar, the model can explain the phenotypes of all tested copy up mutants in these two hosts. Analogous studies were also performed in Salmonella typhimurium and Acetobacter xylinum. The data obtained in these studies indicate that the above model is probably generally true for gram-negative bacteria, and the results also indicate that the maximum tolerable ***copy*** ***number*** is surprisingly low in some hosts.

L18 ANSWER 9 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1994:126459 BIOSIS
DN PREV199497139459
TI Isolation and characterization of DNA-binding mutants of a plasmid replication initiation protein utilizing an in vivo binding assay.
AU Cereghino, Joan Lin; Helinski, Donald R.; Toukdarian, Aresa E. (1)
CS (1) Cent. Molecular Genetics, Univ. Calif. San Diego, San Diego, La Jolla, CA 92093-0634 USA
SO Plasmid, (1994) Vol. 31, No. 1, pp. 89-99.
ISSN: 0147-619X.
DT Article
LA English
AB An in vivo screen was developed for the identification of mutants of the RK2 replication initiation protein, ***TrfA*** , that were altered in their binding to the iterons within the plasmid RK2 origin of replication. This assay is based on an antibiotic selection system originally described by Elledge, Sugiono, Guarente, and Davis (Proc. natl. Acad. Sci USA 86, 3689-3693, 1989) for the isolation in vivo of genes encoding sequence-specific DNA-binding proteins. A ***TrfA*** -specific binding site consisting of two 17-bp iterons separated by a nonrandom 6-bp spacer was placed 3' to a strong constitutive promoter. This promoter-iteron fragment was then inserted into the assay vector convergent to the aadA gene such that an increased level of spectinomycin resistance by the Escherichia coli host was dependent on the binding of wild-type ***TrfA*** protein to the binding site. The in vivo system was used to specifically isolate ***TrfA*** mutants which were either defective in binding or capable of effecting increased levels of spectinomycin resistance as compared to wild-type ***TrfA*** . The defective ***TrfA*** mutants isolated by this screen were purified and found to be considerably less effective in DNA binding by in vitro gel mobility shift assays. The map location was determined for these six defective ***TrfA*** mutants. Each of the mutations consisted of a single base change and mapped within codons extending over a 162 amino acid sequence. All of the mutants which were capable of effecting increased levels of spectinomycin resistance in the in vivo DNA-binding assay also showed some alteration in RK2 replication in vivo with most of the mutants having a copy-up phenotype similar to previously isolated ***TrfA*** mutants able to maintain an eight-iteron RK2 origin plasmid at a higher ***copy*** ***number*** .

L18 ANSWER 10 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1994:31989 BIOSIS
DN PREV199497044989
TI Mutations in the gene encoding the replication-initiation protein of plasmid RK2 produce elevated copy numbers of RK2 derivatives in Escherichia coli and distantly related bacteria.
AU Fang, Ferric C.; Durland, Ross H.; Helinski, Donald R. (1)
CS (1) Cent. Mol. Genet., Dep. Biol., Univ. Calif., San Diego, La Jolla, CA 92093-0634 USA
SO Gene (Amsterdam), (1993) Vol. 133, No. 1, pp. 1-8.
ISSN: 0378-1119.
DT Article
LA English
AB Mini-replicons of the broad-host-range plasmid RK2 with increased ***copy*** ***number*** (cn) due to mutations in the gene encoding the essential replication initiation protein ***TrfA*** are described. The cn of these derivatives have been determined in Escherichia coli, Pseudomonas aeruginosa and Agrobacterium tumefaciens and were found to be elevated in all three bacterial hosts. One of the cn mutations was introduced into the intact 60-kb RK2 plasmid by homologous recombination in vivo, resulting in an approximately twofold cn increase. The expression of ***trfA*** from this mutant RK2 plasmid did not respond to the cn change as predicted by a simple transcription rate-limitation, replication control model. Implications for the model of RK2 replication control and the potential use of mutant RK2 mini-replicons as high-copy broad-host-range gene cloning vectors are discussed.

L18 ANSWER 11 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1991:500897 BIOSIS
DN BA92:123857
TI BROAD-HOST-RANGE PROPERTIES OF PLASMID RK2 IMPORTANCE OF OVERLAPPING GENES
ENCODING THE PLASMID REPLICATION INITIATION PROTEIN ***TRFA*** .
AU FANG F C; HELINSKI D R
CS CENTER MOLECULAR GENETICS DEP. BIOL., UNIV. CALIFORNIA SAN DIEGO, LA JOLLA, CALIF. 92093-0634.
SO J BACTERIOL, (1991) 173 (18), 5881-5888.
CODEN: JOBAAY. ISSN: 0021-9193.
FS BA; OLD
LA English
AB The ***trfA*** gene, encoding the essential replication initiation protein in the broad-host-range plasmid RK2, possesses an in-frame overlapping arrangement. This results in the production of ***TrfA*** proteins of 33 and 44 kDa, respectively. Utilizing deletion and site-specific mutagenesis to alter the ***trfA*** operon, we compared the replication of an RK2-origin plasmid in several distantly related gram-negative bacteria when supported by both ***TrfA*** -44 and ***TrfA*** -33, ***TrfA*** -33 alone, or ***TrfA*** -44/98L (a mutant form of the ***TrfA*** -44 protein) alone. ***TrfA*** -44/98L is identical to wild-type ***TrfA*** -44 with the exception of a single conservative amino acid alteration from methionine to leucine at codon 98; this alteration removes the translational start codon for the ***TrfA*** -33 protein. ***Copy*** ***number*** and stability were virtually identical for plasmids containing both ***TrfA*** -44 and ***TrfA*** -33 proteins or ***TrfA*** -44/98L alone in Pseudomonas aeruginosa and Agrobacterium tumefaciens, two unrelated bacteria in which ***TrfA*** -33 is poorly functional. This, along with recent in vitro studies comparing ***TrfA*** -44, ***TrfA*** -33, and ***TrfA*** -44/98L, suggests that the functional activity of ***TrfA*** -44 is not significantly affected by the 98L mutation. Analysis of minimal RK2 derivatives in certain gram-negative baterial hosts suggests a role of the overlapping arrangement of ***trfA*** in facilitating the broad host range of RK2. RK2 derivatives encoding ***TrfA*** -44/98L alone demonstrated decreased ***copy*** ***number*** and stability in Escherichia coli and Azotobacter vinelandii when compared with derivatives specifying both ***TRfA*** -44 and ***TrfA*** -33. A strategy employing the ***trfA*** -44/98L mutant gene and in vivo homologous recombination was used to eliminate the internal translational start codon of ***trfA*** in the intact RK2 plasmid. The mutant intact RK2 plasmid produced only ***TrfA*** -44/98L. A small reduction in ***copy*** ***number*** and .beta.-lactamase expression resulted in E. coli, suggesting that overlapping ***trfA*** genes also enhance the efficacy of replication of the intact RK2 plasmid.

L18 ANSWER 12 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1991:295027 BIOSIS
DN BA92:16042
TI ISOLATION AND PROPERTIES OF TEMPERATURE-SENSITIVE MUTANTS OF THE TRF-A GENE OF THE BROAD HOST RANGE PLASMID RK2.
AU VALLA S; HAUGAN K; DURLAND R; HELINSKI D R
CS UNIGEN, CENT. MOL. BIOL., UNIV. TRONDHEIM, BROCHS GT. 6, 7030 TRONDHEIM, NORWAY.
SO PLASMID, (1991) 25 (2), 131-136.
CODEN: PLSMDX. ISSN: 0147-619X.
FS BA; OLD
LA English
AB Two small plasmid RK2 derivatives, pSV6 and pSV16, were constructed and used for the isolation and characterization of ***trfA*** mutants temperature-sensitive (ts) for replication in Escherichia coli. Four of the mutants were examined for their ability to initiate replication from the RK2 replication origin in E. coli when present in cis with respect to the origin and in trans when present on a multicopy pBR322 replicon. Each of the mutant ***trfA*** genes exhibited temperature-sensitivity in supporting replication from the RK2 origin when present in cis, and the lowest nonpermissive temperature varied depending on the mutant. When the mutant ***trfA*** genes were present on the multicopy replicon (in trans), three of the four mutant genes could support replication of the RK2-oriV plasmid pSV16 at all temperatures tested. However, with the exception of one of the mutants, the activity was reduced when compared to wild-type. The increased activity in trans possibly is the result of the increased cellular level of the ***TrfA*** protein when compared with the in cis situation where the mutant ***trfA*** gene is at a much lower ***copy*** - ***number*** . Two of the mutants also were tested in cis for temperature sensitivity in Pseudomonas aeruginosa. One of the mutants did not exhibit temperature sensitivity under the conditions employed. The second mutant showed some temperature sensitivity but the nonpermissive temperature pattern was different than that found in E. coli.

L18 ANSWER 13 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1991:180540 BIOSIS
DN BA91:95289
TI ITERON INHIBITION OF PLASMID RK2 REPLICATION IN-VITRO EVIDENCE FOR INTERMOLECULAR COUPLING OF REPLICATION ORIGINS AS A MECHANISM FOR RK2 REPLICATION CONTROL.
AU KITTELL B L; HELINSKI D R
CS DEP. BIOLOGY CENTER MOLECULAR GENETICS, M-034, UNIVERSITY CALIFORNIA SAN DIEGO, LA JOLLA, CALIF. 92093-0634.
SO PROC NATL ACAD SCI U S A, (1991) 88 (4), 1389-1393.
CODEN: PNASA6. ISSN: 0027-8424.
FS BA; OLD
LA English
AB The broad-host-range plasmid RK2 and its derivatives are maintained in Gram-negative bacteria at a specific ***copy*** ***number*** that appears to be determined by a series of direct repeats (iterons) located at the RK2 replication origin and by the RK2 replication initiation protein, ***TrfA*** . An in vitro replication system was developed from Escherichia coli that is active with either the intact eight-iteron RK2 origin or a minimal five-iteron RK2 origin when purified ***TrfA*** protein is provided. Using this in vitro replication system, we have examined the mechanism(s) of ***copy*** - ***number*** control. It was found that two or more RK2 iterons present on a supercoiled compatible plasmid molecule are capable of specifically inhibiting in vitro the replication of either functional RK2 origin plasmid and that this inhibition is not overcome by adding increasing amounts of ***TrfA*** protein. A mutant ***TrfA*** protein, ***TrfA*** -33(cop254D), that increases the ***copy*** ***number*** of an RK2 origin in vivo exhibits replication kinetics and activity levels in this in vitro system similar to that of the wild-type protein. However, RK2 in vitro replication initiated by ***TrfA*** -33(cop254D) has a much reduced sensitivity to iteron inhibition. These data support a model for RD2 ***copy*** - ***number*** control that involves intermolecular coupling between ***TrfA*** -bound iterons.

L18 ANSWER 14 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 1990:375291 BIOSIS
DN BA90:61972
TI MUTATIONS IN THE TRF-A REPLICATION GENE OF THE BROAD-HOST-RANGE PLASMID RK2 RESULT IN ELEVATED PLASMID COPY NUMBERS.
AU DURLAND R H; TOUKDARIAN A; FANG F; HELINSKI D R
CS DEP. BIOL., UNIV. CALIF., SAN DIEGO, LA JOLLA, CALIF. 92093.
SO J BACTERIOL, (1990) 172 (7), 3859-3867.
CODEN: JOBAAY. ISSN: 0021-9193.
FS BA; OLD
LA English
AB Mutated forms of ***trfA*** , the replication protein gene of plasmid RK2 , that support a minimal RK2 origin plasmid in Escherichia coli at copy numbers up to 23-fold higher than normal have been isolated. Six such high- ***copy*** - ***number*** (copy-up) mutations were mapped and sequenced. In each case, a single base transition led to an amino acid substitution in the ***TrfA*** protein primary sequence. The six mutations affected different residues of the protein and were located within a 69-base-pair region encoding 24 amino acids. Dominance tests showed that each of the mutants can be suppressed by wild-type ***trfA*** in trans, but suppression is highly dependent on the amount of wild-type protein produced. Excess mutant ***TrfA*** protein provided in trans significantly increased the ***copy*** ***number*** of RK2 and other self-replicating derivatives of RK2 that contain a wild-type ***trfA*** gene. These observations suggest that the mutations affect a regulatory activity of the ***TrfA*** replication protein that is a key factor in the control of initiation of RK2 replication.

L18 ANSWER 15 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1990:375290 BIOSIS
DN BA90:61971
TI REPLICATION OF THE BROAD-HOST-RANGE PLASMID RK2 DIRECT MEASUREMENT OF INTRACELLULAR CONCENTRATIONS OF THE ESSENTIAL ***TRFA*** REPLICATION PROTEINS AND THEIR EFFECT ON PLASMID ***COPY*** ***NUMBER*** .
AU DURLAND R H; HELINSKI D R
CS DEP. BIOL., UNIV. CALIFORNIA, SAN DIEGO, LA JOLLA, CALIF. 92093.
SO J BACTERIOL, (1990) 172 (7), 3849-3858.
CODEN: JOBAAY. ISSN: 0021-9193.
FS BA; OLD
LA English
AB The ***trfA*** gene of the broad-host-range plasmid RK2 is essential for initiation of plasmid replication. Two related ***TrfA*** proteins of 43 and 32 kilodaltons (kDa) are produced by independent translation initiation at two start codons within the ***trfA*** open reading frame. These proteins were overproduced in Escherichia coli and partially purified. Rabbit antisera raised against the 32-kDa ***TrfA*** protein (***TrfA*** -32) and cross-reacting with the 43-kDa protein (***TrfA*** -43) were used in Western blotting (immunoblotting) assays to measure intracellular ***TrfA*** levels. In logarithmically growing E. coli HB101, RK2 produced 4.6 .+-. 0.6 ng of ***TrfA*** -32 and 1.8 .+-. 0.2 ng of ***TrfA*** -43 per unit of optical density at 600 nm (mean .+-. standard deviation). On the basis of determinations of the number of cells per unit of optical density at 600 nm, this corresponds to about 220 molecules of ***TrfA*** -32 and 80 molecules of ***TrfA*** -43 per cell. Dot blot hybridizations showed that plasmid RK2 is present in about 15 copies per E. coli cell under these conditions. Using plasmid constructs that produce different levels of ***TrfA*** proteins, the effect of excess ***TrfA*** on RK2 replication was tested. A two- to threefold excess of total ***TrfA*** increased the ***copy*** ***number*** of RK2 about 30%. Additional increases in ***TrfA*** protein concentration had no further effect on ***copy*** ***number*** , even at levels 170-fold above normal. An RK2 minimal origin plasmid showed a similar response to intracellular ***TrfA*** concentration. These results demonstrate that ***TrfA*** protein concentration is not strictly rate limiting for RK2 replication and that a mechanism that is independent of ***TrfA*** concentration functions to limit RK2 ***copy*** ***number*** in the presence of excess ***TrfA*** .

L18 ANSWER 16 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1987:462241 BIOSIS
DN BA84:107681
TI NARROW-HOST-RANGE INC-P PLASMID PHH-502-1 LACKS A COMPLETE INC-P REPLICATION SYSTEM.
AU SMITH C A; THOMAS C M
CS DEP. GENETICS, UNIV. BIRMINGHAM, PO BOX 363, BIRMINGHAM B15 2TT, UK.
SO J GEN MICROBIOL, (1987) 133 (8), 2247-2252.
CODEN: JGMIAN. ISSN: 0022-1287.
FS BA; OLD
LA English
AB Plasmid pHH502-1 shows incompatibility only towards members of the IncP group, but has a narrower host range than typical members of that group. In contrast to other IncP plasmids its replication was not affected by a high- ***copy*** - ***number*** plasmid carrying the replication origin (oriV) of IncP plasmid RK2. Southern blotting of pHH502-1 revealed homology to oriV, consistent with its incompatibility phenotype, but no homology to ***trfA*** , the essential replication gene of RK2. Thus it is probable that pHH502-1 does not possess a functional IncP replication system, accounting for its restricted host range. A restriction map of pHH502-1 was constructed and the mercury-resistance determinant was localized to Tn735, which also carries the trimethoprim-resistance determinant and is related to Tn21. The presence of a korB-like function on pHH502-1 was also demonstrated.

L18 ANSWER 17 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1987:209454 BIOSIS
DN BA83:107084
TI CONTROL THE KIL-A GENE OF THE BROAD-HOST-RANGE PLASMID RK-2 INVOLVEMENT OF
KOR-A KOR-B AND A NEW GENE KOR-E.
AU YOUNG C; BURLAGE R S; FIGURSKI D H
CS CANCER CENTER, COLL. PHYSICIANS AND SURGEONS, COLUMBIA UNIV., NEW YORK, N.Y. 10032.
SO J BACTERIOL, (1987) 169 (3), 1315-1320.
CODEN: JOBAAY. ISSN: 0021-9193.
FS BA; OLD
LA English
AB Broad-host-range plasmid RK2 encodes several different kil genes which are potentially lethal to an Escherichia coli host. The kil genes and the essential RK2 replication gene ***trfA*** are regulated by the products of kor genes. We have shown previously that kilA can be controlled by a constitutively expressed korA gene. In this study, we have found that the wild-type, autoregulated korA gene is insufficient for control of kilA cloned on high- ***copy*** - ***number*** plasmids. One of two other genes must also be present with korA. One gene is korB, originally discovered by its ability to control the determinants in the kilB region and later found to affect expression of both ***trfA*** and korA. The other is a new gene, korE, which has been cloned from the 2.2' to 4.1' region located between korC and kilA. Studies with a kilA-cat fusion suggest that korA, korB, annd korE all participate in the control of kilA gene expression.

L18 ANSWER 18 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1988:377630 BIOSIS
DN BA82:72606
TI CONSTRUCTION OF A NOVEL GENE BANK OF BACILLUS-SUBTILIS USING A LOW ***COPY*** ***NUMBER*** VECTOR IN ESCHERICHIA-COLI.
AU HASNAIN S; THOMAS C M
CS DEPARTMENT OF BOTANY, NEW CAMPUS, PUNJAB UNIVERSITY, LAHORE-20, PAKISTAN.
SO J GEN MICROBIOL, (1988) 132 (7), 1863-1874.
CODEN: JGMIAN. ISSN: 0022-1287.
FS BA; OLD
LA English
AB Low ***copy*** ***number*** vector plasmid pCT571 was constructed to clone Bacillus subtilis genomic fragments in Escherichia coli. pCT571 confers KmR, TcR and CmR in E. coli and CmR in B. subtilis. It has unique restriction sites within the KmR and TcR markers to allow screening for recombinant plasmids by insertional inactivation of these genes. It contains the pSC101 replicon and replicates normally at six to eight copies per chromosome equivalent in E. coli. It also contains oriVRK2, which when supplied with the product of the ***trfA*** gene of RK2 in trans, allows pCT571 to replicate at 35-40 copies per chromosome equivalent. A B. subtilis gene bank was created by cloning partially Sau3A-digested and size-fractionated fragments of B. subtilis chromosomal DNA into the BamHI site of pCT571. DNA from 1097 KmR TcS transformants was extracted and analyzed electrophoretically as supercoiled DNA and after digesting with EcoRI or EcoRI and SalI. Approximately 1000 hybrid plasmids were found with reasonably sized B. subtilis fragments. The mean size of the inserts in pCT571 is 8kb, ranging from 4 to 20 kb in different plasmids. The gene bank covers most of the B. subtilis chromosome, as demonstrated by the results of screening the gene bank for selectable nutritional markers in E. coli and B. subtilis. Hybrid plasmids which complement E. coli mutants for arg, his, lys, met, pdx, pyr and thr markers were identified from the gene bank. In B. subtilis the presence of argC, cycA, dal, hisA, ilvA, leuA, lys, metB, metC, phe, purA, purB, thr and trpC was established by transformation experiments. The effects of ***copy*** ***number*** on cloning and long-term maintenance in the bacterial strains were also investigated. A high ***copy*** ***number*** some hybrid plasmids cannot be maintained at all, while others show an increased rate of structural deletions and rearrangements.

L18 ANSWER 19 OF 21 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1985:232577 BIOSIS
DN BA79:12573
TI THE KOR-B GENE TO BROAD HOST RANGE PLASMID RK-2 IS A MAJOR ***COPY*** ***NUMBER*** CONTROL ELEMENT WHICH MAY ACT TOGETHER WITH TRF-B BY LIMITING TRF-A EXPRESSION.
AU THOMAS C M; HUSSAIN A A K
CS DEPARTMENT OF GENETICS, UNIVERSITY OF BIRMINGHAM, P. O. BOX 363, BIRMINGHAM B15 2TT, UK.
SO EMBO (EUR MOL BIOL ORGAN) J, (1984) 3 (7), 1513-1520.
CODEN: EMJODG. ISSN: 0261-4189.
FS BA; OLD
LA English
AB For replication, plasmid RK2 encodes a vegetative replication origin, oriVRK2, and a gene, ***trfA*** , whose polypeptide product(s) is essential for oriVRK2 activity. The ***trfA*** gene is transcribed as part of a polycistronic operon which also includes kilD. Transcription of this operon is negatively regulated by the products of the trfB/korD/korA and korB loci. Mini replicons previously studied in detail lack the korB locus and have copy numbers significantly higher than RK2 itself. Here it is reported that korB in trans expresses incompatibility towards RK2 replicons either when the korB gene dosage is high or when it is expressed from a strong foreign promoter. This incompatibility can be largely overcome if a ***trfA*** gene which is expressed from a foreign promoter, and is therefore not regulated by korB, is supplied in trans. When korB is introduced in cis to mini RK2 replicons the ***copy*** ***number*** is reduced to within the range estimated for parental RK2. Deletions in the oriVRK2 region which otherwise cause quite large increases in plasmid ***copy*** ***number*** have only a small effect when korB is present in cis. Thus, korB in combination with trfB may be the overriding ***copy*** ***number*** control element in RK2 reducing ***trfA*** expression to levels limiting for replication.

L18 ANSWER 20 OF 21 CAPLUS COPYRIGHT 2003 ACS
AN 1988:69726 CAPLUS
DN 108:69726
TI Nucleotide sequence of the transcriptional repressor gene korB which plays a key role in regulation of the ***copy*** ***number*** of broad host-range plasmid RK2
AU Theophilus, Bimal D. M.; Thomas, Christopher M.
CS Dep. Genet., Univ. Birmingham, Birmingham, B15 2TT, UK
SO Nucleic Acids Research (1987), 15(18), 7443-50
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The product of the korB gene of broad host-range plasmid RK2 is one of .gtoreq.2 proteins which repress transcription of the essential replication gene ***trfA*** . The nucleotide sequence of korB and the properties of its predicted polypeptide product KorB which has a mol. wt. of 39,011 Da are reported. KorB is likely to be a sol. protein with an overall net neg. charge. However, consistent with a role in transcriptional regulation, there is a region with extensive homol. to the .alpha.-helix-turn-.alpha.-helix motif of many DNA-binding proteins. This region shows no significant homol. to equiv. regions of the TrfB protein which is the primary transcriptional repressor of RK2 and which binds to an operator whose half sites show considerable homol. to the half sites of the korB operator.

L18 ANSWER 21 OF 21 CAPLUS COPYRIGHT 2003 ACS
AN 1983:588714 CAPLUS
DN 99:188714
TI Instability of a high- ***copy*** - ***number*** mutant of a

miniplasmid derived from broad host range IncP plasmid RK2
AU Thomas, Christopher M.
CS Dep. Genet., Univ. Birmingham, Birmingham, B15 2TT, UK
SO Plasmid (1983), 10(2), 184-95
CODEN: PLSMDX; ISSN: 0147-619X
DT Journal
LA English
AB Mini-RK2 plasmids pCT460 and pCT461 which contain the oriVRK2, ***trfA*** , and trfB regions of plasmid RK2 in addn. to tetracycline- and kanamycin-resistance determinants, have copy nos. of 17 and 35 copies/chromosome equiv., resp. The difference in copy no. is due to a 56-base-pair deletion in oriVRK2 in pCT461. In Escherichia coli, only pCT461 is markedly unstable in batch culture, whereas both are unstable (although pCT461 is more so) in bacteria on stock plates. The instability of pCT461 in bacteria on stock plates is recA+ dependent and appears to involve loss of plasmid DNA from bacteria rather than selective cell death. After storage of recA+ bacteria carrying pCT461 for a few weeks, the remaining antibiotic-resistant bacteria carry a mixt. of plasmid DNA species, including parental pCT461, transposable element insertion derivs., and, by far the majority, deletion derivs. Apparently, 1 particular plasmid region, which includes the kilD gene (which inhibits plasmid maintenance in the absence of korD, which, however, is present on pCT460 and pCT461), is responsible for this instability in a gene dosage-dependent way. Most of these deletion derivs. are dependent on the pCT461-specified ***trfA*** gene (essential for replication), so that they do not displace pCT461 entirely. Their presence reduces the copy no. of pCT461, thus reducing the instability, and is probably ultimately responsible for pCT461 survival on stock plates. In many bacteria, the same process which gives rise to deletion derivs. may result in degrdn. of plasmid DNA extensive enough to cause loss of pCT461.

=> d his

(FILE 'HOME' ENTERED AT 15:51:26 ON 26 FEB 2003)

FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 15:53:45 ON 26 FEB 2003
L1 351 S TRFA
L2 5371 S T-DNA
L3 1 S L1 AND L2
L4 73 S L2 AND ((LEFT BORDER AND RIGHT BORDER) OR (LB AND RB))
L5 38 DUP REM L4 (35 DUPLICATES REMOVED)
L6 0 S L5 AND TRF
L7 2137 S PROMOTER (3A) (CAMV OR PEA PLASTOCYANIN OR HIGH MOLECULAR WEI
L8 0 S L5 AND L7
L9 0 S L1 AND L7
L10 11 S L5 AND BINARY
L11 2271 S PROMOTER (5A) (CAMV OR PEA PLASTOCYANIN OR HIGH MOLECULAR WEI
L12 0 S L11 AND L1
L13 2 S L1 AND REVIEW
L14 2 DUP REM L13 (0 DUPLICATES REMOVED)
L15 252 S RK2 AND L1
L16 42 S L1 AND COPY NUMBER
L17 21 DUP REM L16 (21 DUPLICATES REMOVED)
L18 21 S L17 NOT L14

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